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Some species of the genus Pythium (section Orthosporangium Fisch.) from the soil, with observations on their cultures.

(With 2 plates.)

Many diseases of our garden-plants are caused by fungi, where the infection is effected by means of the soil. Among them the pythiaceous pathogenes play a leading part. We find them in gardens, in the fields and in the woods. From several patterns from spots in the botanical garden in which summer flowers are cultivated and where they are sown before they are finally transplanted, I succeeded in isolating two species of the genus *Pythium,* to cultivate them in artificial cultures and to follow their development. The two species are: *Pythium megalacanthum* de BARY which was identified by comparison with the cultures of the Centraalbureau voor Schimmelcultures, Baarn, and which had been known more as a saprophyte on various plant remains in humid places, only de BARY describing it as a parasite on prothallia of *Todea africana.* The other species was the little known *Pythium spinosum* SAWADA described as parasite in the seedlings of *Antirrhinum maius.*

The *Pythiums* live as parasites in humid soil, on plant or animal remains in water, or as parasites of plants in the soils or in water (rarely as parasites of animals). They were frequently found in the soil (SPESCHNEW 1895, JENSEN 1912, HARVEY 1925, 1927, BYARS-GILBERT 1919, JONES 1925, RAPER 1928), further as parasites on germinating plants (van HALL 1924, RAMOS 1926, JOHANN, HOLBERT, DICKSON 1926, 1928), on coniferous seedlings (GIFFORD 1911, SPAULDING 1914, HARTHY-HAHN 1919, RATBURN 1922, PIERCE-HARTLEY 1919, MAY-YOUNG 1927, SIBILIA 1928), and besides there exists a vaste literature regarding the distribution and infection of the species *Pythium de Baryanum* and the diseases of potatoes and other cultivated plants caused by lower fungi and in which various *Pythiums* play an important part (ORTON 1909, HAWKINS 1917, 1919, DICKSON 1922, SHAPOVALOV-LINK 1924,

HANSFORD 1924, SANFORD 1922, BERKELEY 1925, SIDERIS 1930, 1931). Some species are parasites of plants and after their decay they live as saprophytes on their remains.

Methods.

The soil samples from various spots of the botanical garden were placed into large Petri dishes and these were filled with distilled water. After two days hyphae appeared growing freely from the soil into the water. The clean ends of the hyphae were cultivated on agar plates. A still better method for isolating these Phycomycetes from the soil is that by means of carrots. Pieces of boiled and sterilized carrots are placed into distilled water on soil samples. After two or three days the carrot is covered with the fibres of the fungus which then, transplanted on agar, give pure cultures. In these water-cultures we can get zoosporangia which can serve for further cultures. Cultures without water are more difficult as they are exposed much to infections by other fungi. When we succeed with such a culture we can follow on the carrot the whole evolution of the fungus, especially the formation of the oogonia and antheridia.

To transplant the hyphae for pure cultures I used distilled water so as to get zoosporangia, and besides agar media on which the oogonia formed. In the cultures I used:

1. Soil agar (1000 ccm water, 10 gr sacharose, 0.2 gr K_2HPO_4, 0·2 gr $MgSO_4$, 0·2 gr NaCl, 0·1 gr $CaSO_4$, 15·2 gr agar, 5 gr $CaCO_3$).

2. Carrot agar (1000 ccm water, 20 gr agar, 100 gr carrots).

3. Corn meal agar after MATTHEWS 1931 (1000 ccm water, 10 teaspoonfuls corn meal, 20 gr agar).

The results with these various culture media on the sexual reproduction of the two species are recorded in the following table:

Species	Culture media			
	1.	2	3	4*)
Pythium megalacanthum	+	+	—	+
Pythium spinosum	—	+	+	+

*) Culture on boiled carrot without water.

The table shous the evolution of the oogonia observed in both species; on carrot without water the oogonia developed in both cases in six or seven days. In agar cultures the evolution from the time of inoculation to the appearance of the first oogonia took four to six days under normal laboratory temperature.

Zoospores can be followed well in distilled water into which we brought pieces of the fibres with sporangia. After some hours zoospores develop.

Morphology and Development of the Fungus.

The mycelium is formed by hyphae, 2—4 μ diam., sometimes stronger, often with irregular, numerous and monopodial ramifications. In pure cultures the mycelium consists of the hyphae with the growth out in a radial direction, and variate in the size and method of branching. The hyphae are but rarely forming septa in young cultures (only in sporangia and oogonia), more frequently they appear in older specimens. In places the hyphae form resting conidia which with their spherical (rarely pyriform) shapes of 5—20 μ in diam. correspond fully to the sporangia from which they differ only by growing directly in the mycelium without forming zoospores. In *Pythium megalacanthum* no conidia are formed. In *P. spinosum* they form in great number even in the tissue of the host, in cultures they develop only in distilled water and then mostly intercalarly, once only they were followed terminally. In their germination we can follow instead of one bag the growth of hyphae on two or three sides.

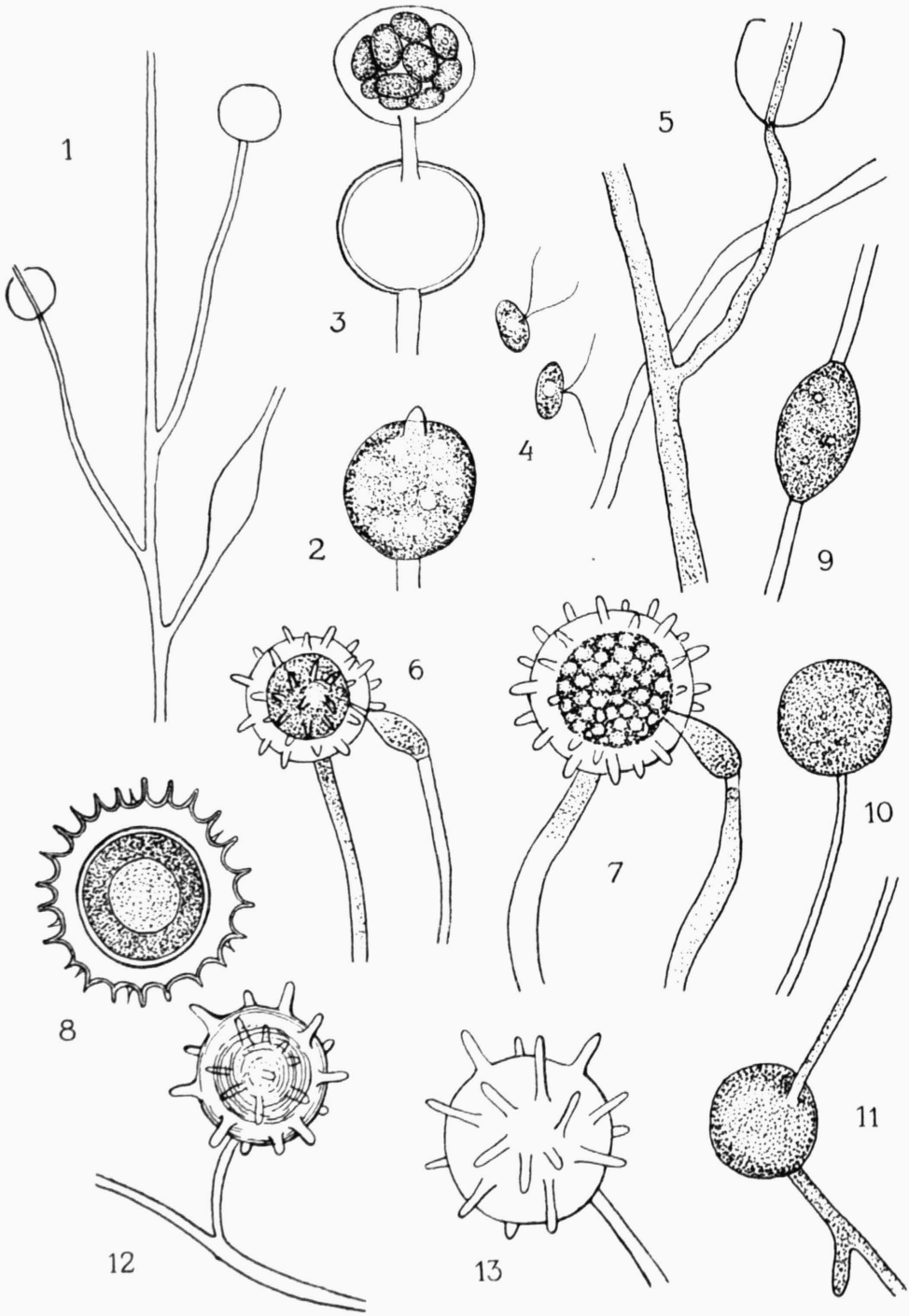
1. Non-Sexual Reproduction.

The generally spherical sporangia develop terminally on short lateral branches, rarely intercalarly with parasitic specimens intramatrically, only on the surface of the epidermis they send out an emptying neck which pierces the surface wall of the body of the host. The size varies: with *Pythium megalacanthum* it is generaly 20—30 μ in diam. These sporangia (presporangia, sporocysts NICOLAS and AGGÉRY 1928, 6) develop a terminal or lateral emptying canal of varying size through which the contents of zoosporangia is emptied out and the walls are still existing, the hyphae continue their growth just as is it the case with the proliferating sporangia of the genus *Saprolegnia, Achlya,* or *Pythium proliferum.* The sporangia are not on sporangiophores which are distinctly separated from vegetative hyphae. The vesicle is a minute, fragile bag into which the contents of the sporangia passes through the duct. The zoospores develop in the middle of the vesicle from 5 to 15 or more, 15 μ in diam. when encysted, with 2 lateral cilia attached on one place (hilum) and they are monoplanetic. Diplanetism was followed only with *Pythium Butleri* and *P. diacarpum* (DRECHSLER 1930, 3, BUTLER 1907, 2), though recently MATTHEWS (1931, 5), maintains that there is here no true diplanetism as it found with *Saprolegnia.*

2. Sexual Reproduction.

The spherical oogonia are sitting terminally (rarely intercalarly) on lateral branches, they are smooth, 35—45 μ in diam. In cultures they soon detach along the septa. In cultures the variations in size observed are between 10 and 50 μ. The walls of the oogonia are covered with long stings varying in length (up to 10 μ long) and crowded. The

I.



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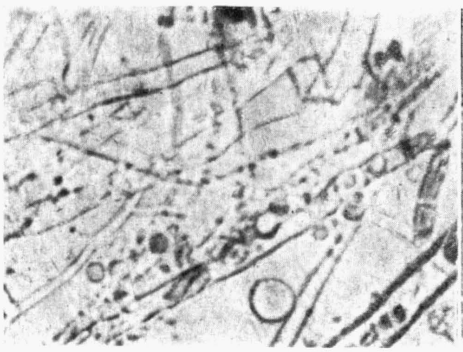

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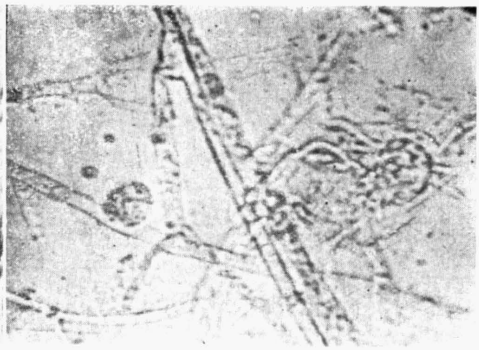

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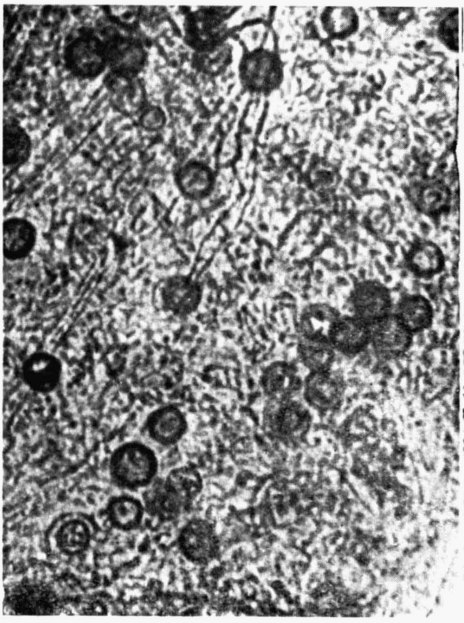

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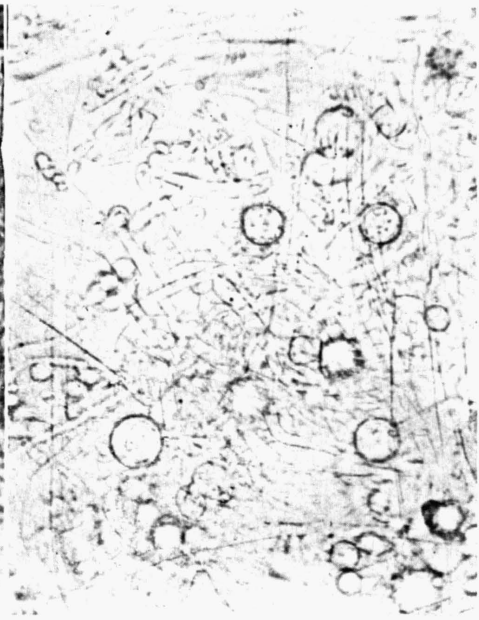

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stings are formed of conical extensions of the membrane. The oogonia contain one big, spherical, and smooth oospore which fills almost the whole interior of the oogonium. With *Pythium spinosum* the oogonia are terminal, spherical, 15—25 μ without the spines, there are very long, sparse spines.

The antheridia grow mostly from the other neighbouring hyphae, generally several around one oogonium. Otherwise also hypogynous or androgynous antheridia are formed of similar hyphae as are those of the oogonia. With *Pythium spinosum* SAWADA (8) describes androgynous antheridia rarely developing from the hyphae below the oogonia. The antheridia appear and differenciate as once as soon as the oogonia begin to develop. The number of antheridia of one oogonium varies, sometimes as many as 25 antheridia were counted.

The process of fecondation was with many species the object of detailled studies from a cytologic point of wiew (PRINGSHEIM, ROZE and CORNU, de BARY, WORONIN, WARD, FISCH, DANGEARD, WAGNER, TROW, BUTLER, MIYAKE, EDSON, PATTERSON, MATTHEWS).

The oospore remain for a long time (several months) within the walls of the oogonia and comes out the breaking down of the walls. Hitherto no germinating oospore has been followed.

Distribution.

Pythium spinosum SAW. — Spines very long, scarce, resting spherical or pear-shaped conidia, antheridia generally androgynous.

It was described by SAWADA as a parasite on seedlings of *Antirrhinum maius*, on Formosa (7, 8). The author experimented also with inoculations of this parasite on other plants (*Allium schoenoprasum, A. fistulosum, Daucus carota, Cucumis sativus, Solanum melongena, Brassica campestris, Chrysanthemum coronarium, Dianthus sinensis, Lactura scariola* var. *sativa*). As remedy against it he recommends wood ashes or a mixture of lime and sulphur with which the fields and the beds in the gardens have to be strewn. In our country it was found in soil in which various annual plants were cultivated, among them also *Antirrhinum maius*. Often I observed the fall of seedlings of *Antirrhinum* in the botanical garden in the hot-beds and also in my own garden. I believe that the cause of this disease was just this parasite.

Pythium megalacanthum de BARY. — Sporangia spherical, separated by a septum, later hyphae proliferating, oogonia with spines, no conidia form.

After de BARY this parasitic fungus did not grow on germinating *Lepidium sativum*, but he was able to cultivate it as a parasite on the prothallia of the fern *Todea africana*. SCHROETER (1889, 9) describes it from rotten stemps of *Veronica hederaefolia*. HARTER and WHITNEY (1927, 4) isolated it from lettuce, BUISMAN (1927, 1), DIDDENS (1931, 2a), from infected roots of flax, in Holland; this di-

sease is called »vlasbrand«. BUISMAN'S observations were confirmed and extended by van der MEER (1928, 10) who experimented with flax-fire. It was found also in the roots of parsley (NICOLAS and AGGÉRY 1928, 6) where very large oogonia were found. In his monograph of the genus *Pythium* of North America MATTHEWS (1931, 5) did not find this species and cites only the observations of european mycologists.

Summary.

1. In soil samples from several spots of the botanical garden *Pythium megalacanthum* de BARY and *P. spinosum* SAW. were found. Both species were isolated and cultivated in different culture media.

2. In distilled water *Pythium spinosum* develops numerous intercalar conidia which often germ simultaneously in two or three hyphae.

3. The presporangia develop a canal through which their contents passes into the vesicle where the zoospores form. After the zoospores have left the sporangium of *P. megalacanthum* continues to grow.

4. The membrane of the extending oogonia form with both species long, sparse spines.

5. *Pythium spinosum* SAW. is a much distributed parasite of the seedlings of summer plants which are cultivated especially in flower-gardens *(Antirrhinum)*, and it causes their fall in spring. Otherwise it lives as a saprophytes in the soil of the beds.

6. *Pythium megalacanthum* de BARY which was formerly described more as a saprophyte, can be likewise such a parasite of garden plants.

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EXPLANATIONS OF FIGURES.

Plate I.

Pythium megalacanthum. 1.—8. — 1. Habit. 2. Mature sporangium just before the formation of a vesicle. 3. Formation of a vesicle with the zoospores. 4. Zoospores. 5. Proliferating sporangium. 6.—7. Oogonia with diclinous antheridium. 8. Optical section of the mature oogonium with oospore.

Pythium spinosum. 9.—13. 9. Intercalary conidium. 10. Terminal conidium. 11. Germinating conidium. 12. Oogonium. 13. Mature oogonium with long spines.

Plate II.

Pythium megalacanthum. 1.—2. — 1. Hyphae, microphotography of culture in soil agar. 2. Antheridia with one oogonium in carrot agar culture.

Pythium spinosum. 3.—4. — 3. Oogonia with mature oospore in corn meal agar culture. 4. Oogonia in carrot agar.